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Predictive model for growth of *Clostridium perfringens* during cooling of cooked ground chicken

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ABSTRACT

Traditional methodologies for development of microbial growth models under dynamic temperature conditions do not take into account the organism's history. Such models have been shown to be inadequate in predicting growth of the organisms under dynamic conditions commonly encountered in the food industry. The objective of the current research was to develop a predictive model for *Clostridium perfringens* spore germination and outgrowth in cooked chicken products during cooling by incorporating a function to describe the prior history of the microbial cell in the secondary model. Incorporating an assumption that growth kinetics depends in an explicit way on the cells' history could provide accurate estimates of growth or inactivation.

Cooked, ground uncured chicken was inoculated with *C. perfringens* spores, and from this chicken, samples were formed and vacuum packaged. For the isothermal experiments, all samples were incubated in a constant temperature water baths stabilized at selected temperatures between 10 and 51 °C and sampled periodically. The samples were cooled from 54.4 to 27 °C and subsequently from 27 to 4 °C at different time periods (cooling rates) for dynamic cooling experiments. The standard model provided predictions that varied from the observed mean $\log_{10}$ growth values by magnitudes up to about 0.65 $\log_{10}$. However, for a selected memory model, estimates of $\log_{10}$ relative growth provided predictions within 0.3 $\log_{10}$ of the mean observed $\log_{10}$ growth values. These findings point to an improvement of predictions obtained by memory models over those obtained by the standard model. More study though is needed to validate the selected model.

Industrial relevance: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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1. Introduction

Clostridium perfringens is an anaerobic spore forming bacterium that is widely distributed in the environment (soil, dust, water and food) as well as the gastrointestinal tract of humans and animals. This wide distribution of the spores has been considered as the main contributing factor for foodborne illness due to *C. perfringens* (McClane, 2001). While the spores can survive for several years in the environment, once germinated, the vegetative cells can replicate rapidly with typical generation times of 7 to 8 min (Labbe & Huang, 1995; Willardsen, Busta, Allen, & Smith, 1978; Willardsen, Busta, & Allen, 1979) at its optimal growth temperature (43–44 °C; Labbe & Juneja, 2006).

C. perfringens is the third most commonly reported bacterial agent of foodborne illness in the United States (Olsen et al., 2000). The organism causes an estimated 250,000 cases of food poisoning annually, leading to about 41 hospitalizations and seven deaths per year in the U.S. (Mead et al., 1999). A majority of the foods implicated in *C. perfringens* foodborne illness were meat and poultry products (Olsen et al., 2000). Improper cooling after cooking and temperature abuse of cooked foods containing meat has been the contributing factor in most of the outbreaks due to *C. perfringens*. The spores of this organism are significantly heat tolerant and can survive the cooking processes applied to most of the processed meats and meat containing foods. The heat treatment can further contribute to the spore germination, resulting in a rapid growth of the organism during improper cooling or temperature abuse during subsequent handling.

The U.S. Food and Drug Administration recommends chilling cooked, potentially hazardous foods from 57 °C to 21 °C within 2 h and subsequently to 5 °C within an additional 4 h (Food Code, 2005) to

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reduce the risk of foodborne illness from spore forming pathogens. Similarly, the USDA Food Safety and Inspection Service (FSIS) compliance guidelines recommend chilling of cooked meat and poultry products from 54.4 to 26.7 °C within 1.5 h and subsequently to 4.4 °C within an additional 5 h (USDA, 2001). Further, the USDA-FSIS stabilization performance standards require that ready-to-eat meat and poultry processors chill the cooked meat and poultry products to control *C. perfringens* spore germination and outgrowth to prevent more than a 10-fold increase in the levels cfu/g during the cooling process.

The objective of this research was to develop a predictive model that could be used to evaluate *C. perfringens* spore germination and outgrowth in uncured chicken products when such products were subjected to changing temperature or temperature abuse conditions. Our primary objective is to characterize growth through the exponential phase, before maximum population densities (MPD) would be reached. This objective is motivated by the regulatory requirement (USDA, 1999) that restricts the growth of *C. perfringens* to lower levels than the maximum population levels, which, in controlled laboratory studies, can typically be $>10^6$ cfu/g.

The general model developed in this paper is similar to the one developed in Juneja, Marks and Thippareddi (2008) for uncured beef. The features of the model include terms that capture an assumed "memory" effect wherein the instantaneous probabilities of changes in the cells' growth phases (lag or exponential phase) depend not only on the instantaneous environment (temperature), but also on the immediately preceding environments. In this paper we consider two such memory models.

The common methodology toward developing a growth model for dynamic environments is to first estimate growth kinetic parameters from a series of growth experiments conducted within specified fixed environments (in this case, isothermal), and from these, develop "secondary models" to determine values for the parameters for any fixed environment within some range. From such models, together with a judiciously selected set of differential equations that describe the growth kinetics or growth change over small increments of time, a general model for predicting relative growth within a changing environment is derived. The coefficients of the differential equations represent instantaneous probabilities of events or hazard functions that dictate the rates of change within cells or the size of a population of cells, and are assumed not to depend on conditions existing before that instant; that is, they are independent of past history. However, it appears from our experiments here and those reported in Juneja et al. (2008) and others reported in the literature (Amézquita, Weller, Wang, Thippareddi, & Burson, 2005) that the standard model did not always provide satisfactory predictions of growth. If history is to be taken into account using these models, one procedure would be to assume that the instantaneous rates or hazard functions at a given time are actually functions of the determined (isothermally) instantaneous rates or hazard functions at times equal to or previous to the given time. One possibility, the approach used in Juneja et al. (2008) and this paper, is to assume that the instantaneous rates at given times are weighted averages of the instantaneous rates derived from isothermal experiments over earlier times. Different formulation of the weighting lead to different predicted $\log_{10}$ relative growth for various cooling scenarios. The selected formulation would be the one that provides the closest predictions to the observed values.

2. Materials and methods

2.1. Test organisms and spore production

Three strains of *C. perfringens*, NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3), and NCTC 10240 (Hobbs serotype 13), were obtained from the Microbial Food Safety Research Unit culture collection (Wyndmoor, PA). *C. perfringens* spores were produced in a modified formulation of the Duncan and Strong sporulation medium, as described previously (Juneja, Call, & Miller, 1993a). The spore crop of each strain was washed twice and then resuspended in sterile distilled water. The suspensions were stored in a refrigerator at 4 °C. The spore population was heat-shocked for 20 min at 75 °C, serially diluted in 0.1% sterile peptone water (PW) and spiral plated (Model D, Spiral Biotech, Bethesda, MD) in duplicate on to tryptose–sulfite–cycloserine agar followed by incubation of plates anaerobically for 48 h at 35 °C. A spore cocktail was prepared immediately prior to experimentation by mixing equal numbers of spores of *C. perfringens* from each of the three suspensions.

Table 1
Growth kinetics estimates for each isothermal growth curve, for Baranyi and linear models

		EGR		LAG		ln (EGR×LAG)	
Temp °C	Replicate	Baranyi	Linear	Baranyi	Linear	Baryani	Linear
13	1	0.04	0.03	198.78	162.44	2.08	1.58
13	2	0.01	0.01	33.43	31.98	−0.74	−0.79
16	1	0.09	0.09	11.75	10.94	0.07	−0.04
16	2	0.09	0.09	12.46	11.76	0.13	0.04
20	1	0.16	0.29	7.33	14.94	0.15	1.45
20	2	0.32	0.31	17.61	17.41	1.72	1.69
23	1	0.65	0.63	11.29	11.20	1.99	1.96
23	2	0.54	0.50	11.11	10.55	1.80	1.66
25	1	0.48	0.48	4.12	4.11	0.69	0.67
25	2	0.43	0.42	4.07	3.98	0.56	0.51
28	1	0.96	0.95	3.26	3.24	1.14	1.13
28	2	0.91	0.87	2.75	2.60	0.92	0.82
31	1	1.06	1.06	2.30	2.30	0.89	0.89
31	2	0.92	0.92	1.54	1.53	0.35	0.35
33	1	0.83	0.82	0.59	0.55	−0.72	−0.80
33	2	1.35	1.33	1.93	1.90	0.95	0.93
35	1	1.39	1.39	1.26	1.29	0.56	0.59
35	2	1.29	1.29	1.56	1.57	0.70	0.71
37	1	1.78	1.83	1.07	1.18	0.64	0.77
37	2	1.81	1.77	1.97	1.93	1.27	1.23
40	1	2.70	2.54	1.45	1.39	1.37	1.26
40	2	1.70	1.67	0.87	0.82	0.39	0.31
43	1	1.95	1.92	0.92	0.89	0.59	0.54
43	2	2.39	2.31	1.12	1.07	0.98	0.90
46	1	2.13	2.18	0.50	0.54	0.07	0.17
46	2	1.96	1.94	0.38	0.37	−0.29	−0.34
48	1	1.76	1.82	0.24	0.30	−0.85	−0.61
48	2	2.15	2.18	0.46	0.49	−0.00	0.06
50	1	1.44	1.52	0.50	0.58	−0.33	−0.12
50	2	1.49	1.42	0.71	0.62	0.05	−0.12
51	1	1.01	0.89	1.19	1.00	0.18	−0.11
51	2	0.67	0.73	0.00	0.00		

2.2. Preparation and inoculation of sampling times and bacterial enumeration

Ground chicken was obtained from a local retail store and frozen (−5 °C) until used (~40 d). The day before the experiment, the ground chicken was thawed overnight in a refrigerator (~4 °C). Ground chicken samples (5-g) were then aseptically weighed into sterile, low-oxygen transmission Whirl Pak bags (4-oz/120-ml capacity; 3″W×7 1/4″L; 7.5 cm×18.5 cm; barrier film 0.125 cc oxygen transmission per 100-inch square in 24 h; Part# B01298WA; Nasco, Modesto, CA) and inoculated with 0.1 ml of the *C. perfringens* spore cocktail to a final concentration of ~3.0 $\log_{10}$ spores/g.

The contents of the bags were thoroughly mixed manually to ensure an even distribution of the spores in the chicken sample. Negative controls included bags containing chicken samples inoculated with 0.1 ml of PW without the bacterial spores. The bags were compressed into a thin layer (ca. 1 mm, thick) by pressing against a flat surface, excluding most of the air, and then heat sealed under vacuum (1000 mbars) using a Multivac vacuum packager (Model A300/16, Multivac Inc., Kansas City, MO). The vacuum packaged samples were

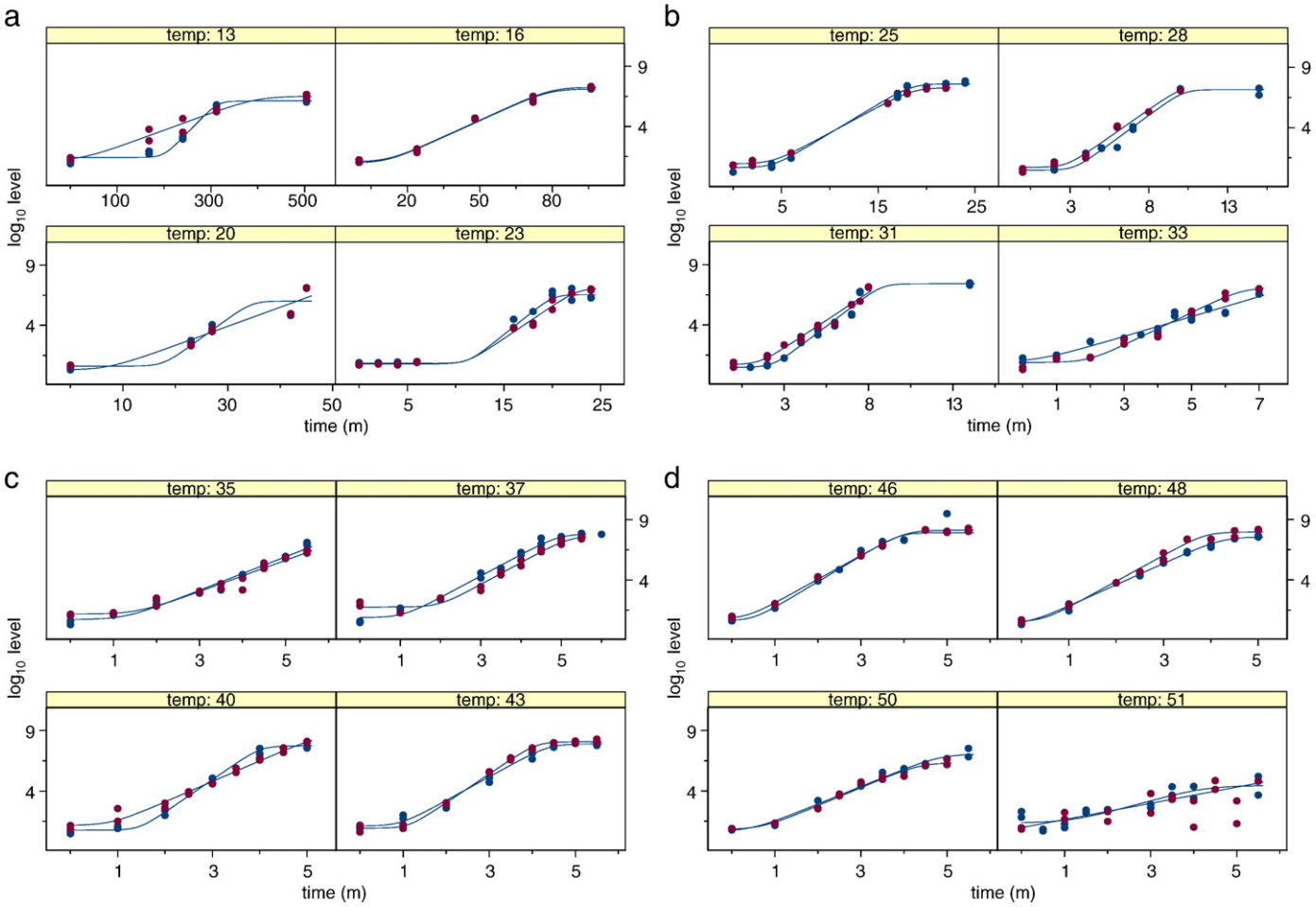

Fig. 1. a–d. Plots of observed $\log_{10}$ levels (cfu/ml) and fitted Baranyi growth curves (Eq. (1)) for isothermal experiments, for each temperature (given in °C) and each replicate experiment.

heat-shocked at 75 °C for 20 min in a water bath; two bags were then opened, chicken in each bag was serially diluted in PW, and then surface plated with a Spiral plater (Model D, Spiral Biotech, Bethesda, MD) on tryptose-sulfite-cycloserine (TSC) agar as described previously (Juneja and Marmer, 1998).

The total *C. perfringens* populations were determined after 48 h incubation at 37 °C in a Bactron anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, OR). This was recorded as the initial inoculated number of bacterial spores after heat-shocking, i.e., time 0. Both non-inoculated raw chicken and heat-shocked chicken (5 g) were used to verify the absence of *C. perfringens* in the ground chicken. Typical black colonies were subjected to further examination using lactose-gelatin and nitrate-motility medium (Schwab et al., 1984).

Thereafter, for the isothermal experiments, all samples were incubated in constant temperature water baths stabilized at selected temperatures between 10 and 51 °C (Table 1). Two independent experiments/replications were done at each temperature. Two bags for each replicate were then removed at designated time intervals, with the sampling frequency based on growth temperature, where the total number of sampling times was about 6–7 for each temperature. The samples were analyzed for total *C. perfringens* populations as described above. For each experiment, an average cfu/g of four platings (analysis of two bags) of each sampling point were recorded and used to determine estimates of the growth kinetics.

For the dynamic cooling experiments, the water bath was programmed so that the temperature decreased linearly with time, between 54.4 °C and 27 °C for a specified duration, and from 27 °C to 4 °C for another specified duration. The samples were stabilized at 54.4 °C or 27 °C, as the case may be, for 10 min before initializing program. Thus, for modeling changing temperatures, it was assumed that the derivative of temperature, $dT(t)/dt = -k$ for some value of k.

2.3. Statistical methods

Procedures for determining a growth model follow those presented in Juneja et al. (2008). Below is a summary of the procedures used.

2.3.1. Primary growth model

A Baranyi function (Baranyi and Roberts, 1994) used to estimate growth at time t was:

$$n(t) = n(0) + \mu A(t) - \ln\left(1 + \frac{e^{\mu A(t)} - 1}{e^{(m-n(0))}}\right) + \varepsilon(t) \quad (1)$$

where

$$A(t) = t + \mu^{-1} \ln\left(\frac{e^{-ut} + q}{1 + q}\right) \quad (2)$$

$n(t)$ is the natural log of the observed level of *C. perfringens* at time t, $\varepsilon(t)$ is an error term for measurements at time t, and μ, q, and m are parameters that determine or affect the exponential growth rate, the lag phase, and the curvature of the growth curve when the population of cells approaches stationary phase. The parameter m is the natural log of the maximum population density, MPD. The equations for

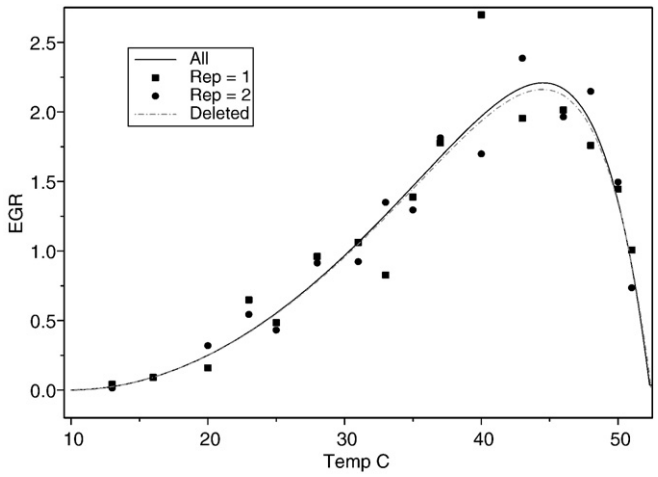

Fig. 2. Estimated exponential growth rates, EGR for Baranyi model, and fitted Ratkowsky model for EGR, versus temperature (Eq. (6)). Solid line is fitted curve when using all data (indicated by ALL); dotted line is fitted curve when deleting EGR value=2.7 sat 40 °C. The model used in the paper was derived using all the data.

exponential growth rate in log-base 10 units (EGR) and the population lag phase duration parameter (LAG) are:

$$\begin{aligned} &\mathrm{EGR} = \mu/\ln(10) \\ &\mathrm{LAG} = \mu^{-1}\ln\left(1+q^{-1}\right). \end{aligned} \tag{3}$$

The product of EGR and LAG is a function of one parameter, q. In Baranyi and Roberts (1994) this parameter reflects the initial "physiological state" of the cells and is often assumed to be constant when samples for the experiments are prepared in a controlled or standard fashion.

A linearized version of the above model is also considered because of the high variability of the estimated maximum population density. Using graphs of the fitted Baryani curves and the observed log levels, data points that appear to represent stationary or near stationary phases of the growth cycle were deleted. A two-compartment, growth model that assumes cells are either in the lag phase or the exponential phase of growth was estimated using the non-deleted data points. The function for this model (Baranyi and Pin, 2001) is:

$$n(t) = \ln\left(\mu e^{-\lambda t} + \lambda e^{\mu t}\right) - \ln(\mu+\lambda) \tag{4}$$

where μ and λ are parameters that determine EGR and LAG, from

$$\begin{aligned} &\mathrm{EGR} = \mu/\ln(10) \\ &\mathrm{LAG} = \mu^{-1}\ln(1+\mu/\lambda) \end{aligned} \tag{5}$$

The product of EGR and LAG is a function of one parameter, $q=\lambda/\mu$, which has the same interpretation as given above for the Baryani growth curve.

2.3.2. Secondary model

The estimated values of the parameters, as a function of temperature, T, were used to estimate secondary models for EGR and LAG. For the EGR, a Ratkowsky (McMeekin, Olley, Ross, Ratkowsky, 1993) equation:

$$\mathrm{EGR}^{1/2} = a(T-T_{\min})[1-\exp(b(T-T_{\max}))]^{1/2} \tag{6}$$

was used, where $T_{\min}$ and $T_{\max}$ are the minimum and maximum temperatures for which within this range of temperature there are non-zero EGR values, and a and b are parameters. To determine the LAG, for a given value of EGR, the natural log of the product, $\zeta=\ln(\mathrm{EGR}\times\mathrm{LAG})$ is considered. The variable ζ was assumed to be a function of temperature; parameter values of the function were estimated by statistical analysis. For estimating parameter values of Eq. (6) and ζ, a mixed effect model, assuming a nested variance structure, was used to determine the statistical significance of a non-zero between-replicate, within-temperature, variance component.

To determine predicted growth for changing temperature, the following differential equations (Juneja et al., 2003b, 2008) are used:

$$\begin{aligned} &\frac{dm_0(t)}{dt} = -h(t)m_0(t) \\ &\frac{dm_D(t)}{dt} = h(t)m_0(t) + \mu(t)m_D(t)\left(1-\frac{m_0(t)+m_D(t)}{M}\right) \end{aligned} \tag{7}$$

where $h(t)$ is the hazard function for cells in the lag phase (=λ when constant), $\mu(t)$ is the exponential growth rate expressed in natural log terms, $m_O(t)$ is the level of cells in the lag phase, and $m_D(t)$ is the level of cells in the exponential phase, and M is the maximum population density. The effect of the "logistic" term on the right of the second equation above is miniscule on the growth during the lag and the initial exponential phases of growth. The dynamic parameters $h(t)$ and $\mu(t)$ are computed from the secondary model Eq. (5), where temperature $T(t)$ is a known function of time.

In Juneja et al. (2008) a model was developed that assumed an effect of previous temperatures on the hazard functions that would carry forward in some fashion. These were called memory-of-Δ models, in which integrated hazards, $\tilde{h}(t)$ and $\tilde{\mu}(t)$ where $\tilde{h}(t)=\int w_h(s)h(s)ds$ and $\mu(t)=\int w_u(s)h(s)ds$, and w_h and w_μ are functions, defined below. These are, respectively, substituted for $h(t)$ and $\mu(t)$ in Eq. (7). For the lag times, it was assumed a weighting function, $w_h(s)=6(s-t)(t-\Delta-s)/\Delta^3$, for s in the interval $I=[\max(0,\ t-\Delta),\ t]$, zero elsewhere. The integral of this weighting function is 1, and the function is symmetric in the interval $[t-\Delta,\ t]$ when $t>\Delta$. Thus using instead of $h(t)$ "shifts" the hazard function by about $\Delta/2$. For the exponential growth rate hazard function, $\mu(t)$, the usual assumption is that μ is unaffected by previous environments; however, we can image that there is some "carry-over" effect, though perhaps not as great as the effect incurred for the lag times. Thus, instead of a weighting function, $w_h(s)$, for $\tilde{\mu}(t)$, the weighting function, $w_\mu(s)$, was defined such that it is at its maximum at t. A weighting function $w_\mu(s)=3(t-\Delta-s)^2/\Delta^3$ satisfies this property. In some of the memory models, Δ was not assumed constant but rather was assumed to be a decreasing function of the exponential growth rate. This property is assumed based on the notion that the carry over effect assumed for these memory models would depend upon the speed of the cell's metabolic mechanisms regarding processing nutrients and preparing itself for eventual cell

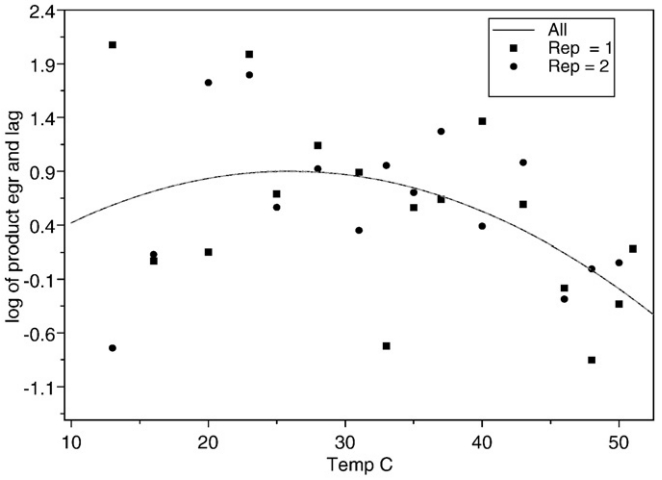

Fig. 3. Estimated values of $\xi=\ln(\mathrm{EGR}\times\mathrm{LAG})$ (y-axis) versus temperature (°C) for the Baranyi model, where solid line is the estimated quadratic regression with independent variable equal to temperature, derived using all the data.

Table 2
Estimates of values for secondary model parameters for exponential growth rate, EGR and $\zeta = \ln(\text{EGR} \times \text{LAG})$

Model	Growth temp °C		a	b	c	d	e
	Max	Min					
Baranyi	52.14	9.61	0.047	0.217	−0.39	0.10	−0.0019
Linear	52.07	9.68	0.047	0.228	−0.41	0.10	−0.0019

The models are: $\text{EGR}^{1/2} = a(T - T_{\min})[1 - \exp(b(T - T_{\max}))]^{1/2}$, $\zeta = c + dT + eT^2$ where T is temperature °C.

division: the more rapid this processing the less memory carry-over, and thus smaller value of Δ. The value of EGR provides a measure which is assumed to have a positive correlation with the cell's metabolic processing rates. Two functions are considered: $F_1(\mu) = 1 - \mu(t)/8$, and $F_2(\mu) = 1 - \mu(t)^{1/2}/8$.

To determine the variance error matrix of the estimated parameters of these secondary equations and of estimates of $\log_{10}$ relative growth from these equations, bootstrap estimation was used. For each temperature, there were 2 growth experiments. These are assumed to be random selections from a population of all such experimental growth experiments at that temperature. For each bootstrap, from the two experiments at each temperature, two randomly selected experiments with replacement were made, and from these the estimates of the parameter values defining the secondary equations were made. The variance error matrix and confidence intervals were estimated from 2500 bootstraps. That is, the standard deviation of the 2500 generated values of a parameter is the standard error of the estimated parameter value, and the complete error variance matrix is determined from the covariance matrix of the bootstrapped parameter values over the 2500 bootstrap realizations.

Estimates of parameter values of equations were made using linear, non-linear, and mixed effect regression procedures of the SAS software system (SAS, 2004), using default options (there was no need to adjust any of options). Graphs were constructed using S-PLUS®. Statistical comparisons between models (goodness of fit) were made by likelihood ratio tests based on differences of $L = -2$ log-likelihood values, which is provided as an output of the SAS procedure. The significance of the difference between two values of L was approximated as a chi-square distribution with degrees of freedom equal to the difference in the number of parameters used in fitting the model (assuming one model is directly obtainable from the other model by eliminating a subset of parameters or assuming fixed values for them).

3. Results

3.1. Preliminary examination of the raw data

One data point, at 46 °C, replicate 1, and 5.0 min, was deleted, since the estimated $\log_{10}$ level of 9.48 was 1.18 $\log_{10}$ greater than the next highest value of 8.30 (which was at a different temperature) and 1.30 $\log_{10}$ greater than its replicate. Further, 2 data points at 51 °C, replicate 2, one at 4 min and one at 5 min, were deleted because their $\log_{10}$ measured values were 1.0 and 1.3, respectively, nearly 2 $\log_{10}$ less than the surrounding values, and both had residuals exceeding 2.5 $\log_{10}$ in the initial regression analysis. At 10 °C there was no observed growth.

3.2. Primary growth model

Fig. 1a–d provides graphs of the fitted Baranyi growth curves of Eq. (1) and a plot of all data (including those data that were deleted from the analysis) for each temperature from 13 °C to 51 °C. Table 1 provides estimates of EGR and LAG for both the Baryani model (Eq. (3)) and the linear model (Eq. (4)) obtained by eliminating data points that were judged to be in the stationary or near stationary phase, for each growth experiment excluding the ones at 10 °C. Note that the estimated EGR value at 40 °C for the first replicate appear to be unexpectedly large, relative to neighboring values.

3.3. Secondary model

3.3.1. EGR

The influence of the data point at 40 °C on the estimated parameter values of the Ratkowsky equation (Eq. (6)) was minimal; however, the model-predicted EGR values differed from the estimated EGR values given in Table 1 by 0.71 for the Baryani model and 0.57 for the linear model. Consequently, the data point was deleted from the analysis. Fig. 2 is a plot of the EGR estimated values for the linear model together with the predicted EGR from the fitted Ratkowsky equation. The model parameters were estimated with $\ln(\text{EGR}^{1/2})$ as the dependent variable, using nonlinear regression, and an assumed a heteroscedastic standard deviation for the residual standard deviation:

$$\sigma_r = fe^{\rho(T - T_{\min})} \tag{8}$$

The estimate of ρ was −0.0322, with a standard error of 0.00954, and was statistically significant at the 0.002 level. The estimate of f was 0.207, with a standard error of 0.055. The value of σ_r is approximately 1/2 the CV of EGR. Using this approximation, 20.7% would be an

Table 3
Mean values and estimated standard errors and correlations of parameter values for the exponential model given in Table 2, based on 2500 bootstraps

Variable	Temp		a	b	$\ln f$	$\ln -r$	c	d	e	$\ln(h)$	$\ln(-\varphi)$
	Max	Min									
Mean	52.09	9.56	0.0471	0.234	−1.634	−3.275	−0.035	0.077	−.0016	0.502	−3.354
SD	0.20	0.51	0.0020	0.045	0.348	0.373	0.815	0.048	0.0007	0.331	0.266
Temp max	1.00	0.46	0.6190	−0.862	−0.385	−0.264	−0.137	0.176	−.2066	−0.420	−0.342
Temp min	0.46	1.00	0.8835	−0.690	−0.348	−0.220	−0.799	0.790	−.7779	−0.201	−0.111
a	0.62	0.88	1.0000	−0.857	−0.579	−0.422	−0.664	0.722	−.7472	−0.439	−0.264
b	−0.86	−0.69	−.8573	1.000	0.615	0.465	0.333	−0.407	0.4578	0.602	0.472
$\ln(f)$	−0.38	−0.35	−.5792	0.615	1.000	0.908	0.013	−0.108	0.1676	0.678	0.503
$\ln(-\rho)$	−0.26	−0.22	−.4217	0.465	0.908	1.000	−0.068	−0.006	0.0572	0.574	0.462
c	−0.14	−0.80	−.6642	0.333	0.013	−0.068	1.000	−0.985	0.9609	−0.024	−0.082
d	0.18	0.79	0.7216	−0.407	−0.108	−0.006	−0.985	1.000	−.9937	−0.083	0.014
e	−0.21	−0.78	−.7472	0.458	0.168	0.057	0.961	−0.994	1.0000	0.164	0.054
$\ln(h)$	−0.42	−0.20	−.4386	0.602	0.678	0.574	−0.024	−0.083	0.1644	1.000	0.901
$\ln(-\varphi)$	−0.34	−0.11	−.2644	0.472	0.503	0.462	−0.082	0.014	0.0536	0.901	1.000

The estimated standard error for a parameter value is equal to the standard deviation of the 2500 bootstrapped results for the parameter. Heteroscedastic standard deviation for the residual standard deviations of $\ln(\text{EGR}^{0.5})$: $\sigma_r = fe^{\rho(T - T\min)}$, and for ζ: $\sigma_r = he^{\varphi T}$ as a function of temperature were assumed. The natural logarithms of the temperature coefficient ρ, and f were used because the distributions of these transformed variables were more symmetric than those of the untransformed variables.

Table 4
Predictions of, and observed, $\log_{10}$ increase for dynamic growth curves with constant rate (linear) decline in temperature between the designated temperatures

Time (h) between		Mean initial $\log_{10}$ level	Predicted $\log_{10}$ increase		Observed $\log_{10}$ increase		Difference (observed-linear)
54.4 and 27 °C	27 and 4 °C		Baranyi	Linear		Std. dev.	
			Models				
1.5	0.0	2.81	1.14	1.15	0.47	0.05	−0.68
1.5	12.5	2.27	3.24	3.22	2.39	0.06	−0.83
1.5	15.0	2.38	3.66	3.64	3.54	0.25	−0.09
3.0	0.0	2.28	3.32	3.33	2.73	0.39	−0.60
3.0	7.5	2.49	4.55	4.54	4.13	0.25	−0.42
3.0	10.0	2.96	4.74	4.73	2.67	0.13	−2.06
3.0	12.5	2.49	5.16	5.15	3.79	0.91	−1.36
4.5	0.0	2.33	5.26	5.27	4.73	0.25	−0.54

Each observed value is the average of observed results ($\log_{10}$) from two growth experiments.

estimate of 1/2 the CV of EGR at or near the minimum temperature; as the temperature increases, the CV decreases; near the maximum temperature, where 1/2 the CV of EGR was estimated to be about 5.3%, with a standard error of about 1.1%.

3.3.2. LAG

Fig. 3 is a plot of the estimates of $\xi = \ln(\mathrm{EGR} \times \mathrm{LAG})$ for the exponential model versus temperature, together with a linear regression line. The assumption of assuming that ξ is constant is not supported by the plots. In addition the residual standard deviations appear to be homoscedastic. Linear regression was performed assuming that ξ is a quadratic in temperature and that the residual standard deviations can be described as:

$$\sigma_r = he^{\varphi T} \tag{9}$$

where h and φ are constants. Thus there are 5 parameters in this model; the between replicate, within temperature, variance component was not significant.

3.3.3. Summary of secondary model

Table 2 presents the estimates of the values of the parameters, a, b, T_{max} and T_{min}, identified in Eq. (6) for determining EGR and the coefficients, c, d, e, in the quadratic equation in temperature, for determining ζ. Using the parameter values of Table 2, predictions of $\log_{10}$ relative growth can be obtained for any temperature. Table 3 provides the mean values of the 2500 bootstrap estimates of the parameter values for the linear model, their standard deviations (which are the estimates of the standard errors of the estimated parameter values), and the correlations among the estimated variables. The distributions of the bootstrap realizations for the estimated values of the parameters were nearly normal, with the exception of the parameters f, ρ, h and φ of Eqs. (8) and (9). For these

Table 5
Standard errors of estimated selected $\log_{10}$ relative growths from the linear model, based on 2500 bootstraps

Time between 54.4 and 27	Time between 27 and 4	Average observed $\log_{10}$ relative growth	Std. dev.	Mean $\log_{10}$ relative growth linear model	Std. error $\log_{10}$ relative growth linear model
1.5	0.0	0.47	0.05	1.15	0.065
1.5	12.5	2.39	0.06	3.25	0.124
1.5	15.0	3.54	0.25	3.67	0.137
3.0	0.0	2.73	0.39	3.33	0.110
3.0	7.5	4.13	0.25	4.55	0.122
3.0	10.0	2.67	0.13	4.73	0.077
3.0	12.5	3.79	0.91	5.15	0.087
4.5	0.0	4.73	0.25	5.25	0.096

Table 6
Predictions, using memory — Δ h — models for exponential model, with Δ=0, 0.25 h and 0.5 h, of $\log_{10}$ relative growth for selected cooling scenarios with temperatures declining at a constant rate between the given endpoint temperatures

Hours from		Mean observed	Predicted $\log_{10}$ relative growth for linear model				
54.4 to 27 °C	27 to 4 °C		Δ=0 h	Δ=0.25 h	Δ=0.5 h	F1	F2
1.5	–[a]	0.47	1.15	1.01	0.87	0.98	0.76
1.5	12.5	2.39	3.22	3.12	3.02	3.24	3.00
1.5	15.0	3.54	3.64	3.53	3.43	3.65	3.41
3.0	–[a]	2.73	3.33	3.21	3.07	3.11	2.96
1.5	5.0	–	1.97	1.87	1.76	1.98	1.75

In addition, two functions of EGR for Δ are considered: $F1 = 1 - (\mathrm{EGR})\ln(10)/8$, and $F2 = 1 - [\mathrm{EGR}\ln(10)]^{1/2}/8$.
[a] Experiment stopped at 27 °C.

parameters, a natural log transformation was used: e.g., $\ln(f)$ and $\ln(-\rho)$, which provided more normal distributions. The standard error estimates obtained from the bootstrap were either nearly equal the ones obtained from the regression analyses, or slightly larger. For example, for T_{max} the mean value of the bootstrap iterations was 52.087, with a standard deviation of 0.200, versus 52.071 obtained from the regression, with a standard error of 0.184. From the bootstrap estimates, the lower 99% confidence bound of T_{min} was 8.42 °C, and the upper 99% confidence bound was 10.60 °C; for T_{max}, the lower 99% confidence bound was 51.69 °C, and the upper 99% confidence bound was 52.56 °C. These are quite near the estimates obtained, using the regression results and normal approximation, of 51.62 °C and 52.52 °C, based on 31 degrees of freedom.

3.4. Predictions for dynamic cooling scenarios

Growth experiments were performed where temperatures changed linearly from 54.4 °C to 27 °C at one rate, and then at another rate, from 27 °C to 4 °C. Experiments thus are labeled as (h_1, h_2) where h_1 is the hours for cooling from 54.4 °C to 27 °C, and h_2, from 27 °C to 4 °C. Table 4 gives predicted and observed $\log_{10}$ relative increases of levels for the Baryani and linear models for various cooling scenarios studied. The differences among the estimates of relative growth for the two models were quite insignificant. Generally, while the populations of cells were in the exponential phase of growth, the model-predicted relative growths were larger than the observed relative growths. Standard errors of the linear model estimated $\log_{10}$ relative growth are given in Table 5 for the exponential and logistic models, based on the bootstrap of 2500 iterations.

To see the effect of incorporating a memory, calculations of $\log_{10}$ relative growth are given for fixed values of Δ, and for when Δ is a function of the EGR. Table 6 gives predicted $\log_{10}$ relative growth for selected scenarios for the exponential model with Δ=0.25 h and 0.5 h, and for two functions: $F_1(\Delta) = 1 - [\mathrm{EGR}(t)\ln(10)]/8$, and $F_2(\Delta) = 1 - [\mathrm{EGR}(t)\ln(10)]^{1/2}/8$. When Δ is constant, the predictions are lower than the corresponding predictions for the no-memory (Δ=0 h) model. When the function was equal to F_2, differences of the predictions of observed $\log_{10}$ relative growths from the observed values were less than 0.3 $\log_{10}$ in magnitude, and seemed to provide the best predictions among the models considered for the scenarios studied. For the Food Safety and Inspection Service (FSIS) compliance guidelines cooling scenario, (1.5, 5), the estimate obtained assuming Δ=0 was 1.97 $\log_{10}$ relative growth, while using memory model with F_2 the estimate was 1.76. These estimates exceed the present USDA (1999) requirement of no more than 1 $\log_{10}$ relative growth.

4. Conclusion

For this study, the growth of *C. perfringens* in uncured chicken was explored. The values of growth model parameters were derived using

the usual approach: first developing primary growth equations in isothermal environments, then developing secondary equations derived from estimated primary growth parameter values. Using the parameter values for the secondary equations, and a set of differential equations that describe the instantaneous behavior of growth kinetic parameters, relative growth of *C. perfringens* can be estimated for any cooling scenario. The secondary equations were based on a Ratkowsky curve for determining the exponential growth rate, EGR, as a function of temperature. To estimate the lag phase duration, an assumption often made is that the products of the population lag phase and EGR are the same, independent of temperature, reflecting the physiological conditions of the cells. However, these data were not consistent with this assumption; rather, for the models of this paper, it was assumed that the logarithmic transformation of the product was a quadratic polynomial in temperature.

The estimated range of temperatures for growth was about 52 °C to 9.7 °C. For a temperature decline, linearly, from 54.4 °C to 27 °C in 1.5 h, the standard model predicted a $\log_{10}$ relative growth of about 1.15, while the mean of the observed $\log_{10}$ relative growth results for two replicates was 0.47 $\log_{10}$; for the same temperature decline in 3 h, the predicted $\log_{10}$ relative growth was about 3.33 $\log_{10}$ and the mean observed $\log_{10}$ relative growth was 2.73 $\log_{10}$. For a cooling scenario that extends to 4 °C, of 54.4 °C to 27 °C, linearly, in 1.5 h and 27 °C to 4 °C, linearly, in 12.5 h, the average observed and predicted $\log_{10}$ relative growths were 2.73 $\log_{10}$ and 3.22 $\log_{10}$, respectively; when cooling was extended from 27 °C to 4 °C, linearly, in 15 h, the average observed and predicted $\log_{10}$ relative growths were 3.62 $\log_{10}$ and 3.64 $\log_{10}$, respectively. For the latter cooling scenario the levels were greater than 6 $\log_{10}$, still less than stationary levels of about 7 or 8 $\log_{10}$.

The standard model for predicting growth in dynamic cooling scenarios is based on the appropriateness of translating, directly, results obtained for isothermal environments into differential equations with coefficients that assumed to be representing rates of instantaneous changes of cell states (hazard functions), dependent only on time, with no-memory. This assumption is certainly not innocuous; it assumes the past experience of cells would not have an impact on the cells' processing mechanisms so as to not affect their specific rates of growths at any time. To introduce memory, the standard model was adjusted by incorporating another parameter, Δ, where it is assumed that the hazard functions of a cell leaving the lag phase and entering the exponential phase of growth, or of a cell dividing once in the exponential phase of growth, depend on environments (temperatures) occurring earlier, from $t-\Delta$ to t. Specifically, hazards functions that are used in the differential equations that reflect the growth dynamics were assumed to be weighted integrals, from $t-\Delta$ to t, of the isothermal derived hazard functions that are used in the standard model. In addition, the length of memory, Δ, was assumed to be a decreasing function of the exponential growth rate. When the function was equal to $1-[\mathrm{EGRln}(10)]^{1/2}/8$, differences of the predictions of observed $\log_{10}$ relative growths and the observed values were not more than 0.3 $\log_{10}$ in absolute value.

More research to validate the above types of model adjustments is needed. However, the information in this paper presents a model which can be used to design cooling processes to help ensure the safety of ready-to-eat chicken products.

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